from a shad fish captured from the Taiwan Strait gave limits of detection for the organochlorine pesticides in fish below ng g^{-1}. The concentration of *p*,*p*′-DDE was detected at 90 ng g^{-1}.

Future Prospects

In recent years, new and improved methods and technologies to analyse pesticide residues have rapidly evolved. Analytical methods for most residue pesticides have detection and quantification limits at the low parts per billion to parts per trillion for water analysis, and low parts per million to parts per billion for other samples such as crops, soils and biological matrices. Many preconcentration methods have been developed in clean-up procedures, including exhaustive solvent extraction, automated Soxhlet extraction, microwave-assisted extraction, MSPD extraction, SPE using microcolumns, cartridges, and Empore discs, and SPME. Qualitative and quantitative determination by GC with element-selective detectors, and confirmation of results using MS, continues to be the predominant technique for multiresidue pesticide analysis. MS has been widely used for confirmation of trace pesticide identification and quantitation. Tandem mass spectrometry (MS-MS) is replacing the conventional approaches to confirmation and GC added to the MS-MS will increase the specificity obtained. Now that ion trap mass spectrometry has been introduced, pesticide residue confirmation can be obtained through experiments involving MS-MS or MS^n on compounds of high relative molecular mass, to yield a pyramid of related product ions. With mass spectrometry developments the MS-MS technique will become inexpensive, and eventually the GC-MS-MS technique will be the conventional means of analysing pesticide in most classes of sample matrices.

See also: **II / Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **Extraction:** Analytical Extractions; Solid-Phase Extraction; Solid-Phase Microextraction; Supercritical Fluid Extraction. **III / Herbicides:** Gas Chromatography; Solid-Phase Extraction; Thin-Layer (Planar) Chromatography. **Pesticides:** Extraction from Water; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

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Supercritical Fluid Chromatography

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Introduction

Supercritical fluid chromatography (SFC) is a useful tool in the analysis of pesticides and herbicides. Typically, this is done with liquid or gas chromatography and there are basic advantages and disadvantages to each of these method of analysis. For LC, the liquid phase offers the unique advantage of a wide range of solubilities but detection interfaces tend to be the limiting factor. GC has the ability to be more easily interfaced to specific detection capabilities but has significant limitations in the area of solubility; compounds must be able to be readily volatilized. For agriculturally active compounds, the lack of a universal detector for HPLC limits the scope of its applicability to compounds which do not contain UV chromophores. GC is limited to thermally stable volatile compounds since they must move through the

chromatographic column in the gaseous state. Not all pesticides and herbicides contain a chromophore, others are thermally labile, or are not volatile, and therefore require derivatization for GC analysis. The analysis of pesticides and herbicides by supercritical fluid chromatography is a reasonable alternative to LC and GC. SFC offers the ability to be coupled to a wide variety of detectors that are both LC and GC compatible. In addition, the solubilizing powers of a supercritical fluid at low operating temperatures can easily mimic those achieved with liquids, thus making SFC more applicable to a wider range of pesticide and herbicide classes than either LC or GC.

Since the mid-80s, the advantages of SFC in pesticide and herbicide analysis have been well documented. SFC has also been used to solve separation problems. In the main stream of pesticide and herbicide analysis however, SFC remains a niche technique that is employed when classical LC and GC analysis are not successful in solving a problem. Even in those examples where SFE may be used to remove the herbicidal or pesticidal analyte of interest, the follow-up technique is still predominantly gas or liquid chromatography.

This report outlines typical examples of the use of supercritical fluids for the chromatographic analysis of pesticides and herbicides by pesticide class. **Table 1** lists these pesticides, their common and trade names, mode of action and pesticide class.

Carbamates and Methyl Carbamates

Carbamate pesticides were rapidly analysed with flame ionization detection by Wright and Smith. The compounds investigated were propoxur, chlorpropham, carbaryl, and phenmedipham and baseline resolution was achieved in less than 1.5 min. To achieve this rapid separation, the authors used a short capillary column (0.9 m × 25 μm i.d.) coated with 5% phenyl polymethylsiloxane that had been crosslinked with azo-tert-butane. This type of rapid separation represents one of the other distinct advantages of SFC. The high diffusivities of supercritical fluids allow for rapid equilibration, as the compounds of interest travel through the chromatographic column achieving higher separation factors in less time. Overall analysis times are therefore reduced. In this separation, pure carbon dioxide was used as the mobile phase with pressure programming rates of 100 atm min^{-1}. The baseline resolution of propoxur, dicamba, carbaryl, 2,4-D, silvex, and phenmedipham was achieved later in approximately 120 s by these same authors, again demonstrating the rapid analysis of carbamate pesticides by SFC. For this mixture of six pesticides, the separation was achieved on a 1.5 m × 25 μm, 5% phenyl polymethylsiloxane column with pressure programming at 50 atm min^{-1}. This mixture was further expanded with the addition of picloram and chloramben and baseline resolution was obtained on a 1.5 m capillary column in approximately 120 s, demonstrating consistently the rapid analysis times that can be achieved using SFC for carbamates.

In more typical separation timeframes, Richter reported the separation of four carbamate pesticides on a more standard size column 15 m × 50 μm i.d. fused silica column with an SE-33 stationary phase (0.25 μm film thickness). Aldicarb, methomyl, diflubenzuron, and phenmedipham were baseline resolved in a little more than 20 min.

UV detection at 254 nm was used to analyse carbamate pesticides by Games and co-workers using analytical-scale packed columns with modified carbon dioxide. The pesticides analysed were chlorpropham, pirimcarb, methiocarb, carbaryl, phenmedipham and asulam. Resolution was achieved in approximately 5 min on a 10 cm × 4.6 mm i.d. LiChrosorb column. 12% methanol-modified carbon dioxide was the mobile phase and the flow rate was programmed to help achieve the rapid separation by increasing the rate from 2 mL min^{-1} to 4 mL min^{-1} after the first 2 min.

Although nitrous oxide, especially when mixed with alcohol modifiers, has since been shown to be a safety hazard under supercritical conditions, initially investigators explored the use of nitrous oxide as an alternative to carbon dioxide to obtain alternative separation mechanisms. Capillary SFC for the separation of free amines and their carbamate and amide derivatives was investigated by Mathiasson *et al.* with nitrous oxide as the mobile phase. A thermionic nitrogen–phosphorus detector was used and its performance optimized by systematic variation of the makeup gas flow rate (nitrogen), the hydrogen and air flow rates, and the bead current. The effects of increasing nitrous oxide pressure on detector response were explored and a dependence of peak area on the system pressure was observed. This same effect on detector response was seen with the addition of methanol modifier to the nitrous oxide. Concentrations of methanol above 0.8% resulted in a significant loss in signal from the detector.

Aldicarb, methomyl, mesurol, oxamyl, carbofuran, and carbaryl were analysed by capillary SFC by Richter and coworkers in parsley extracts. The column was a 10 m × 50 μm i.d. SB-methyl-100 and a nitrogen–phosphorus detector was used. Practical limits of detection were achieved, the pesticides were determined in the parsley extract at levels of approximately 2 ppb.

Table 1 Compound classes of herbicides, insecticides and pesticides

Compound common name	*Trade name*	*Mode of action*	*Compound class*
α-BHC or Benzene Hexachloride		Ingested insecticide	Organochlorine insecticide
Alachlor	Lasso™	Cell division inhibitor	2-Chloroacetanilide herbicide
Aldicarb	Temik	Cholinesterase inhibitor	Carbamoyloxime insecticide
Aldrin	Octalene		Organochlorine insecticide
Atrazine	Gesaprim	Photosynthetic electron transport inhibitor	1,3,5-Triazine
β-BHC or Benzene hexachloride		Ingested insecticide	Organochlorine insecticide
Bendiocarb	Ficam, Garvox, Seedox	Cholinesterase inhibitor	Methyl carbamate insecticide
Captafal	Difolatan	Protective non-systemic fungicide	Phthalimide fungicide
Captan	Orthocide™	Protective spray, root or dip fungicide	Phthalimide fungicide
Carbaryl	Sevin	Cholinesterase inhibitor	Methyl carbamate insecticide
Carbofuran	Furadan, Curaterr, Yaltox	Cholinesterase inhibitor	Carbamate insecticide
Carbophenothion	Trithion	Acaricide	Organophosphorus insecticide
Chlorbromuron	Maloran	Photosynthetic electron transport inhibitor	Urea herbicide
Chlordane	Octachlor	Non-systemic contact and ingested insecticide	Chlorinated hydrocarbon insecticide
Chlorpyrifos	Dursban, Lorsban	Cholinesterase inhibitor	Organophosphorus insecticide
DDD	Rhothane		Organochlorine insecticide
DDE	Gesarol, Guersarol™	Non-systemic ingested contact insecticide	Organochlorine insecticide
DDT	Gersarol, Guesarol, Neocid	Non-systemic ingested and contact insecticide	Organochlorine insecticide
Dieldrin	Octalox		Organochlorine insecticide
Diflubenzuron	Dimilin	Chitin synthesis inhibitor	Benzoylurea insecticides
Dioxathion	Delnav		Organophosphorus insecticide
Disulfoton	Di-Syston, Dithiosystox, Frumin AL, Solvirex	Cholinesterase inhibitor	Organophosphorus insecticide
Diuron	Karmex	Photosynthesis inhibitor	Urea
Ethyl parathion	Thiophos, Bladan, Folidol, Fosferno, Niran™	Cholinesterase inhibitor	Organophosphorus insecticide

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Table 1 *Continued*

Compound common name	*Trade name*	*Mode of action*	*Compound class*
Fenchlorphos	Nankor Trolene Korlan	Systemic insecticide	Organophosphorus insecticide
Fenitrothion	Accothion, Cytel, Cyfen, Folithion, Sumithion	Cholinesterase inhibitor	Organophosphorus insecticide
γ-BHC or benzene hexachloride	Lindane, Gammexane	Ingested insecticide	Organophosphorus insecticide
Hexachlorobenzene, HCB	Voronit C	Selective fungicide used to control *Tilletia caries* on wheat	Organochlorine fungicide
Malathion, Carbofos	Cythion	Cholinesterase inhibitor	Organophosphorus insecticide
Metalaxyl	Apron, Ridomil, Fubol	Systemic fungicide	Acetalanine
Methidathion	Supracide Ultracide	Cholinesterase inhibitor	Organophosphorus insecticide
Methomyl	Lannate, Nudrin™	Cholinesterase inhibitor	Carbamoyloxime insecticide
Methoxychlor	Marlate	Contact and stomach insecticide	Bridged-di-phenyl insecticide
Methyl parathion	Trithion	Cholinesterase inhibitor	Organophosphorus insecticide
Metobromuron	Patoran	Photosynthetic electron transport inhibitor	Urea herbicide
Oxamyl	Vydate	Cholinesterase inhibitor	Carbamoyloxime insecticide
Parathion	Thiophos, Bladan, Folidol, Fosferno, Niran	Cholinesterase inhibitor	Organophosphorus insecticide
Pentachlorophenol	Dowacide G, Santobrite	Termite control	Phenolic herbicide, insecticide and fungicide
Phenmedipham	Betanal	Photosynthetic electron transport inhibitor	Bicarbamate herbicide
Phorate	Thimet, Agrimet™	Cholinesterase inhibitor	Organophosphorus insecticide
Tetrachlorvinphos	Gardona, Rabond,	Cholinesterase inhibitor	Organophosphorus insecticide
Tetradifon	Tedion V-18	Non-systemic acaricide	Bridged diphenyl acaricide
Thiodicarb	Larvin, Semevin	Cholinesterase inhibitor	Carbamoyloxime insecticide
Tri-allate	Avadex BW, Far-Go	Cell elongation inhibitor	Thiocarbamate herbicide

The separation of the thermally labile acid and carbamate pesticides propoxur, BPMC (fenobucarb), propachlor, carbofuran, alachlor, carbaryl, linuron, and diuron was achieved by Wright *et al.* using SFC/MS with ammonia chemical ionization. Capillary SFC was used for sample introduction into the MS instrument. Resolution of all compounds except BPMC and propachlor was obtained on

a 10 m × 50 μm 5% phenyl methylpolysiloxane stationary phase. Ammonia provided softer ionization with an (M + 18) + molecular ion and little fragmentation, while methane chemical ionization resulted in an (M + 1) + molecular ion and increased fragmentation when the two reagent gases were compared. These same authors conducted additional work comparing the same reagent gases for the chemical ionization of a larger group of carbamate and acid pesticides. Again, capillary SFC was used as a means of sample introduction. The separated and identified carbamate pesticides were: aldicarb, aldicarb sulfoxide, aldicarb sulfone, carbaryl, BPMC, propoxur, chlorpropham, carbofuran, asulam, desmedipham, and penmedipham. The acid pesticides explored were 2,4-D, 2,3-D methyl ester, dicamba, piclotam, Silvex and Silvex methyl ester. The capillary columns were short (2 m × 50 μm i.d.) the pressure ramps rapid (50 atm min^{-1}). Spectra obtained using ammonia resembled thermospray HPLC/MS spectra. The general rule for carbamates was that the ammonium adduct ion was the base peak. The absence of significant thermal degradation was indicated since the molecular species was present.

Carbofuran, its 3-keto, and 3-hydroxy metabolites extracted from the gullet of a bird were analysed by multidimensional SFC/SFC. A 1.0 μL aliquot of bird extract was injected allowing for detection limits for both metabolites and carbofuran at levels in the range 1 to 10 ng. A flow-switching interface demonstrated the use of two 50-μm-i.d. capillary columns in tandem. In this example, a biphenyl column was first in the series, a glyme column second. The use of solvent venting allowed for the injection of large volumes.

A multichannel UV detector for capillary SFC was used to analyse the pesticides bendiocarb and carbaryl and the herbicides alachlor, diuron and metalaxyl. A photodiode array spectrophotometer acquired the UV spectra. The detector flow cell volume was 710 nL made from a fused silica capillary (0.32 mm i.d.) with the polyimide coating removed. The capillary column a 12 m × 100 μm i.d. 5% phenylmethyl polysiloxane achieved baseline resolution in 31 min. Full spectra over the range 190 to 310 nm were collected at the peak apexes for each compound. Excellent sensitivity was achieved, for example the limit of detection of bendicarb (S/N = 5) was 3.8 ng.

Triazines

Both packed and capillary column SFC have been successfully used for the analysis of triazine herbicides. To achieve good signal/noise ratios at concentrations as low as 5 ppm, Ashraf and coworkers reported using solvent-vented injections of as much as 1.0 μL samples onto a 2 m × 110 μm i.d. retention gap. The gap was connected through a venting valve to a 10 m × 50 μm i.d. fused silica capillary SFC column. The valve was switched to the inject position for a controlled amount of time when as little as 0.2 μL of sample was delivered, or for the full length of the chromatographic run for the delivery of 1.0 μL of sample. During the injection process, the sample loop was purged with nitrogen gas. After injection, the repositioned venting valve passed the flow of nitrogen through to the vent, concentrating the injected compounds on the pre-column. Another switch of the venting valve brought the analytical column in-line for the chromatographic analysis. This injection technique was successful for up to 20 ppm solutions of the two triazine herbicides, atrazine and cyanazine. Two pyrethroids and one benzophenylurea compound were also successfully injected using this technique.

Packed column SFC was used by Taylor *et al.* to investigate the behaviour of triazine and triazole herbicides. Carbon dioxide flow rate, outlet pressure, and oven temperature were explored using an analytical scale, 25 cm × 4.6 mm i.d., Deltabond CN column. Baseline resolution of the eight compounds of interest was obtained in approximately six minutes again illustrating the rapid equilibration and elution possible with supercritical fluids. In an atypical flow delivery method, the flow of carbon dioxide was held constant, while the flow of methanol was increased during the chromatographic run. Over the course of the six minute chromatographic run, the methanol concentration was increased from 2.4% to approximately 30%. The outlet pressure during the separation was 270 atm (4000 psi) and the oven temperature was held constant at 60°C. There is little question that supercritical conditions were not maintained during the analysis. Specifically, once the methanol concentration reached levels at or above 12 to 15%, sub-critical or enhanced fluidity chromatography were the most likely mobile phases affecting the separation. Despite the change in mobile phase state throughout the chromatographic process, the separation was successfully achieved.

Concentration ranges from 2.5 ppb to 25 ppm were examined by Ashraf *et al.* using a 'three electrode' thermionic detector. Both the phosphorus and nitrogen selective modes were explored with capillary SFC as the means of sample introduction. The compounds of interest included two triazine herbicides, two pyrethroids, a benzophenylurea, and a chlorophenyl vinyl diethylphosphone. The capillary SFC column was a 10 m × 50 μm i.d. biphenyl methyl polysiloxane. Significant baseline rise was observed during pressure programming. To reduce this effect,

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optimization experiments for hydrogen, air, and nitrogen flow rates (as makeup gas), and the position of the alkali source in relation to the flame tip were conducted. In the phosphorus mode, detection in the picogram range was demonstrated for the vinyl phosphone. Detection was only slightly improved over what could be obtained with flame ionization detection when nitrogen-containing compounds, such as the triazines, were examined in the phosphorus mode. In the nitrogen mode, the background current was much lower. This did not result in a less severe baseline increase with pressure programming. This detector was found to demonstrate enhanced sensitivity in the nitrogen mode, with detection limits in the range 0.6 to 60 pg.

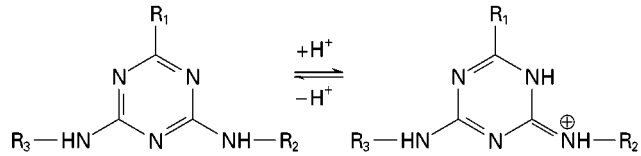
SFC/MS and GC/MS were both easily connected with capillary SFC via a capillary-direct interface. The interface used required no modification of the mass spectrometer. Hawthorne and Miller reported the analysis of a triazine herbicide metabolite using capillary SFC/MS and obtaining spectra collected in the CI mode with methane as the reagent gas.

A minimum detection limit of approximately 35 pg (S/N = 3) was obtained for a triazole fungicide metabolite on a 5 m × 50 μm SB-methyl-100 column. An electron capture detector was utilized for this capillary SFC analysis. Pressure programming from 100 to 350 atm (1500 to 5100 psi) at 40 atm min^{-1} was performed without a substantial increase in detector background signal. Makeup gas of 10% methane in argon was used with a detector operating temperature of 350°C. The optimum conditions for system operation were found with makeup gas flow rate of 15 mL min^{-1}, and the restrictor positioned approximately 3 cm from the column nut at the entrance to the detector.

The effects of 'enhanced fluidity' mobile phases on s-triazines: ammeline, hydroxyatrazine, desethyl-desisopropyl atrazine, atrazine, terbutyne and terbutylazine have been shown. **Figure 1** shows the names, structures and pK_a values of these herbicides. Enhanced fluidity chromatography uses carbon dioxide at lower percentages and mobile phase modifiers such as methanol at higher percentages than SFC. This yields gains in diffusivity and viscosity over liquid chromatography but not to the level obtained with SFC. **Figure 2** shows the chromatographic separation of four of these triazines at elevated pressures and temperatures with three different mobile phase compositions. **Table 2** shows the chromatographic efficiencies and retention times of analytes with different mobile phase conditions. These values are taken from the chromatograms illustrated in Figure 2. Ultimately, for these triazine herbicides the viscosity reduction of the 'enhanced fluidity' mobile phase yielded higher optimum flow rates, higher efficiency, shorter analysis time, and decreased pressure drop. These are the same advantages seen when a supercritical fluid mobile phase is used, but to a lesser extent. The example has been included in this review of SFC to be inclusive and to illustrate to the reader that many examples in the literature which claim to be 'supercritical', fall in and out of the supercritical region of a phase diagram. Even so, advantages over liquid chromatographic separations can be seen.

Ureas and Sulfonylureas

Much work has been carried out using supercritical techniques on ureas and sulfonylureas. This class of herbicides was newly introduced in the early 1980s.



	R_1	R_2	R_3	pK_a
Ammeline	OH	H	H	4.5, 9.4
Hydroxyatrazine	OH	C_2H_5	$CH(CH_3)_2$	4.9
Desethyl-desisopropyl atrazine	Cl	H	H	1.5
Atrazine	Cl	C_2H_5	$CH(CH_3)_2$	1.7
Terbutyne	SCH_3	C_2H_5	$CH(CH_3)_2$	4.4
Terbuthylazine	Cl	C_2H_5	$CH(CH_3)_2$	1.9

Figure 1 Chemical structures and pK_a values of triazine herbicides.

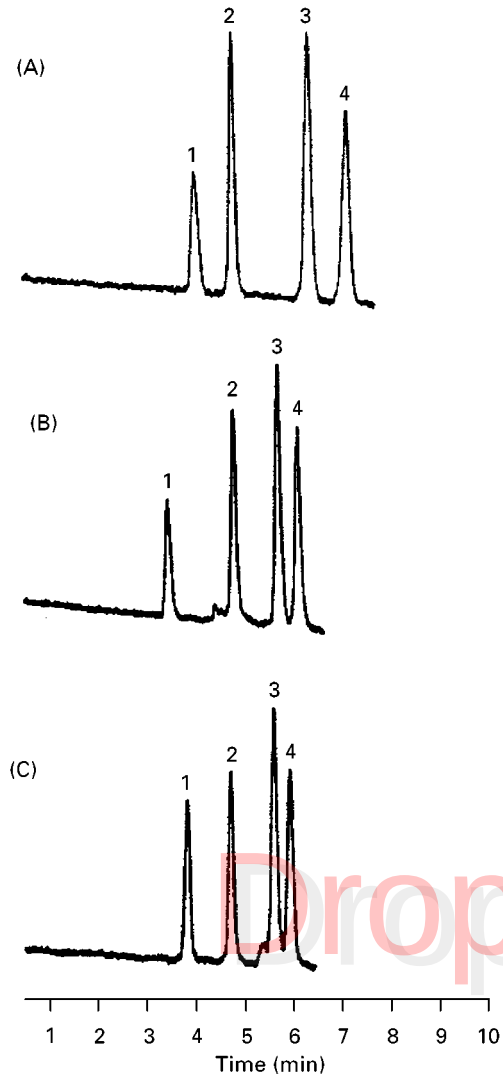


Figure 2 Chromatograms at 238 atm, 0.35 mL min^{-1} for different mobile phase conditions: (A) 64 : 36 mol% methanol–H_2O; (B) 51 : 29 : 20 methanol–H_2O–CO_2; (C) 51 : 29 : 20 methanol–H_2O–CHF_3. Peaks: 1. hydroxyatrazine; 2. desethyl-deisopropyl atrazine, 3. atrazine, 4. terbutylazine.

They have low use rates in the agricultural arena and so require low analytical detection limits and they are thermally labile. The desired low detection limits make liquid chromatography more difficult and the thermal lability make GC inappropriate. Wheeler and McNally compared packed and capillary supercritical fluid chromatography with HPLC using representative ureas and sulfonylureas. Five herbicides were analysed by the three techniques. For both the capillary and the packed column SFC experiments and LC, UV detection was used. Limits of detection, reproducibility, and linearity of response were compared. The compounds investigated were the moderately polar herbicides, Oust®, Glean®, Harmony®, Karmex® and Nustar®. Oust®, Glean® and Harmony® are sulfonyl urea herbicides. Karmex® or diuron is a phenyl methyl urea, and Nustar® is a silicon fungicide. Results indicated that faster analysis, lower detection limits, and greater injection-to-injection reproducibility were obtainable with packed column SFC. No appreciable difference in the linearity of response between the three techniques was observed. Using capillary SFC with FID detection, short capillary columns were used to examine these same compounds. Relative standard deviations for peak area were 3 to 5% range. The linear range was found to be fairly compound dependent, and detection limits were reported in the range 20 to 80 μg mL^{-1}.

Baseline resolution of dimethylcarbanilide, dimethylphenylurea (linuron), diphenylmethylurea (diuron), monuron, and carbanilide was achieved in eight minutes. Shah and Taylor used an analytical scale packed cyanopropyl column. Using a methanol modified carbon dioxide mobile phase, the separation was achieved holding the methanol concentration constant at 2%, and the flow rate at 3 mL min^{-1}. Ureas, sulfonylureas and their manufacturing precursors have been separated using packed and capillary SFC columns, a carbon dioxide mobile phase and an on-line FTIR detector. The column used in the urea separation was a packed 10 cm × 1 mm i.d. cyano column. Capillary columns and split-less injections of 0.1 μL were employed for precursors at concentrations of approximately 3 mg mL^{-1}. The ureas studied were: dimethylphenyl urea, diphenylmethyl urea, monuron, and carbanilide. On the packed column, baseline resolution of all compounds except for the partially resolved dimethylcarbanilide and dimethylphenyl urea, was obtained. Peak assignments were made based on retention time and spectral interpretation comparison with pure standards. After attempts to develop the separation on the packed CN column failed, capillary SFC columns were used to separate the benzamide–anilide mixture. The baseline resolution of six precursors was achieved in approximately 25 min.

The packed SFC retention behaviour of a variety of urea and sulfonyl urea precursors was studied with modified carbon dioxide mobile phase by McNally and co-workers. Methyl, phenyl, nitro, amide, carboxamide and chloro functional group positioning were examined to gain an understanding of retention characteristics. Extensive modifier interactions were investigated in this study, using methanol, ethanol, isopropanol, hexanol and tetrahydrofuran at 2% w/v in carbon dioxide. A silica stationary phase was chosen to simulate polar matrices, i.e. soil and plant materials. Comparisons with supercritical fluid extraction retention were drawn. This was the first

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Table 2 Efficiencies and retention times of analytes with different mobile phase conditions at the same flow rate: 0.35 mL min^{-1}[a]

Peak	Mobile phase (mol%), flow rate					
	64/36 methanol/H_2O, 0.37 mL min^{-1}		51/29/20 methanol/H_2O/CO_2, 0.35 mL min^{-1}		51/29/20 methanol/H_2O/CHF_3, 0.37 mL min^{-1}	
	Efficiency	t (min)	Efficiency	t (min)	Efficiency	t (min)
1	3714	4.05	4271	3.54	4908	3.81
2	8054	4.83	8932	4.80	9254	4.70
3	9672	6.4	10870	5.72	11588	5.59
4	10306	7.2	11332	6.14	11738	5.92

[a]Peak numbers are the same as in Figure 2. The values provided are averages of at least three replicated chromatograms with RSD $\leq$ 5%.

comparison of the two made in the literature. Since that time, several extensive reports have proven this correlation to be true. In this functional group study, specific interactions were observed which were attributed to the polarity of the compound, steric interactions between individual functional groups of the molecules, the functional group molecular make-up, and the polarity of the modifier used.

A benchtop thermospray mass spectrometer has been coupled with packed column SFC for the detection of the packed column SFC separation of ureas. The column used to separate dimethylcarbanilide, dimethylphenyl urea, diphenylmethyl urea, monuron, diuron, and carbanalide was a 1-mm i.d. cyanopropyl column. Significant baseline rise with pressure programming was noted and attributed to hydrocarbon contamination in the carbon dioxide mobile phase. This was eliminated in the selective ion monitoring (SIM) mode of the mass spectrometer.

Diuron was the target analyte in a study which examined the effect of repeller potential on spectra obtained by SFC/MS using a thermospray interface. The influence of the repeller potential on the degree of fragmentation was studied. An analytical scale (4.6 mm i.d.) packed column was used for sample introduction with 2% methanol carbon dioxide mobile phase. Spectra of diuron at low repeller potentials resembled CI spectra while at high repeller potentials the result was close to the EI spectra of diuron. High vaporizer temperature led to the thermal decomposition of diuron.

As has been stated, the advantages of SFC are that separation is achieved rapidly, with a high number of theoretical plates, leading to resolving powers that are far superior to those found in liquid chromatography. The use of SFC for the separation of a wide range of sulfonylurea compounds has been demonstrated in the analysis of environmental water samples. Preceded by a simple concentration step on an SPE cartridge, as low as 0.5 ng ml^{-1} detection levels in the original sample were achieved reproducibly. No additional sample clean-up was required. This was not the case with capillary electrophoresis and liquid chromatography. **Figures 3(A)**, **3(B)** and **3(C)**, show the SFC/UV chromatograms obtained for spiked Milli-Q water, and spiked river and spike creek water respectively. As is illustrated, chromatographic interference was not seen in any of the chromatograms. The SFC conditions coupled six standard 5 μm packed analytical columns in series, one Zorbax® ODS, 4.6 mm × 25 cm, and five Zorbax Silica also 4.6 mm × 25 cm. The mobile phase was a carbon dioxide methanol gradient, initial methanol percentage 1% at 90 bar held constant for four minutes, then ramped to 7% at 150 bar at 10 min, then held at 150 bar but ramped to 16% methanol in an additional ten minutes. The temperature was 60°C, flow rate 2.00 mL min^{-1}. Retention times were in the 30 to 40 minute range. This was quick considering the total length of the six columns. An example chromatogram of twelve separated sulfonylurea compounds is illustrated in **Figure 4**. Again, no interference is seen in the creek water, in the retention time of interest.

Organophosphoros Pesticides

Malathion, phoxim, ethion, dimethoate, and azinphos-methyl in onion and tomato extracts have been separated by packed capillary SFC with a phosphorus-selective thermionic detector. Attempts at separating these compounds with pure carbon dioxide were unsuccessful; methanol and 2-propanol were used as modifiers. 1.5% methanol resulted in baseline resolution in nine minutes. 3.5% 2-propanol also achieved baseline resolution but inferior peak shape. The packed column was a 5 μm C-18 15 cm × 0.32 mm i.d. packed. The detector performance was optimized with regard to mobile phase composition, hydrogen and air flow rates, and the

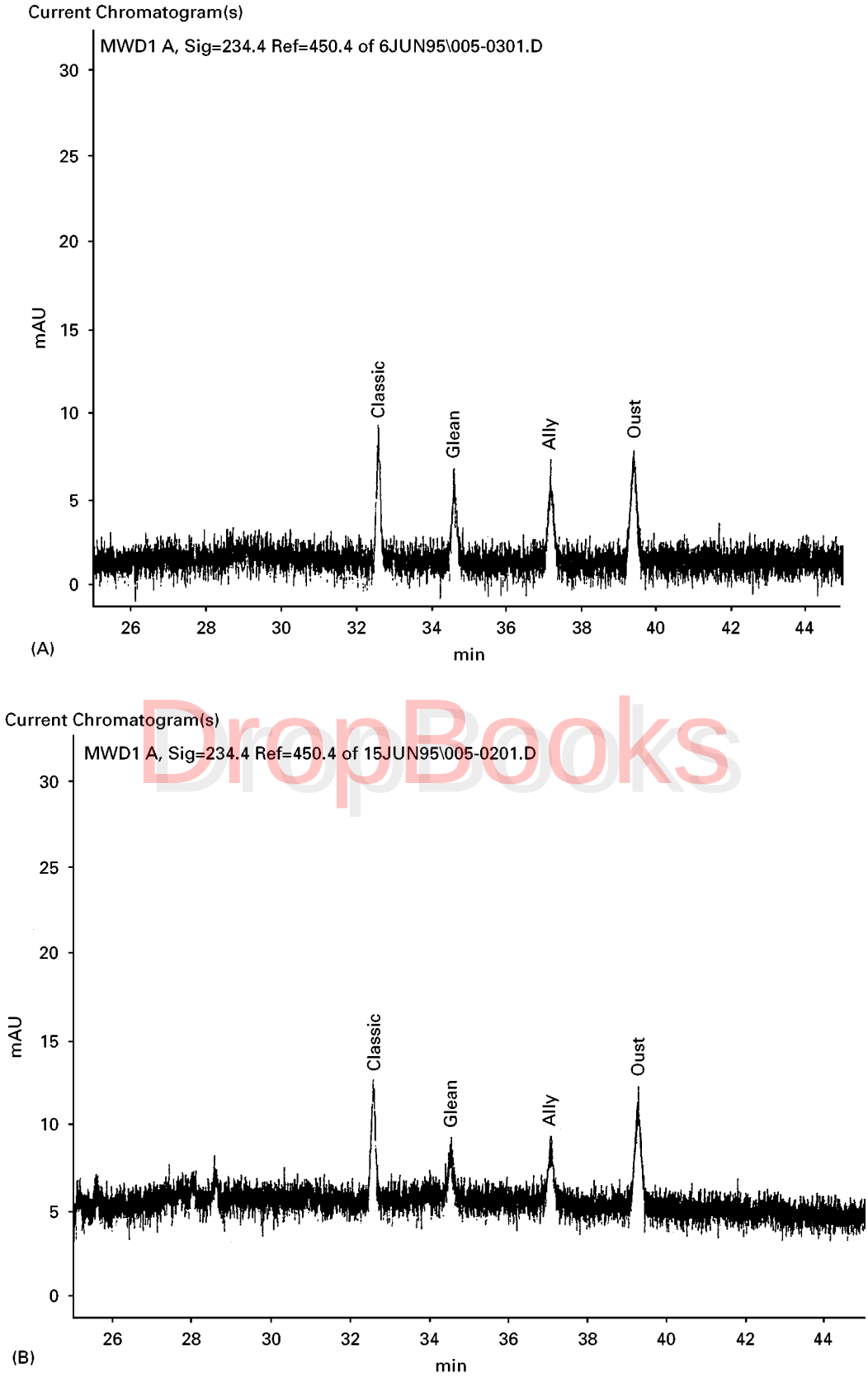

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Figure 3 (A) Millo-O water at 0.5 ppb. (B) White Clay Creek water at 0.5 ppb. (C) Brandywine River water at 0.5 ppb.

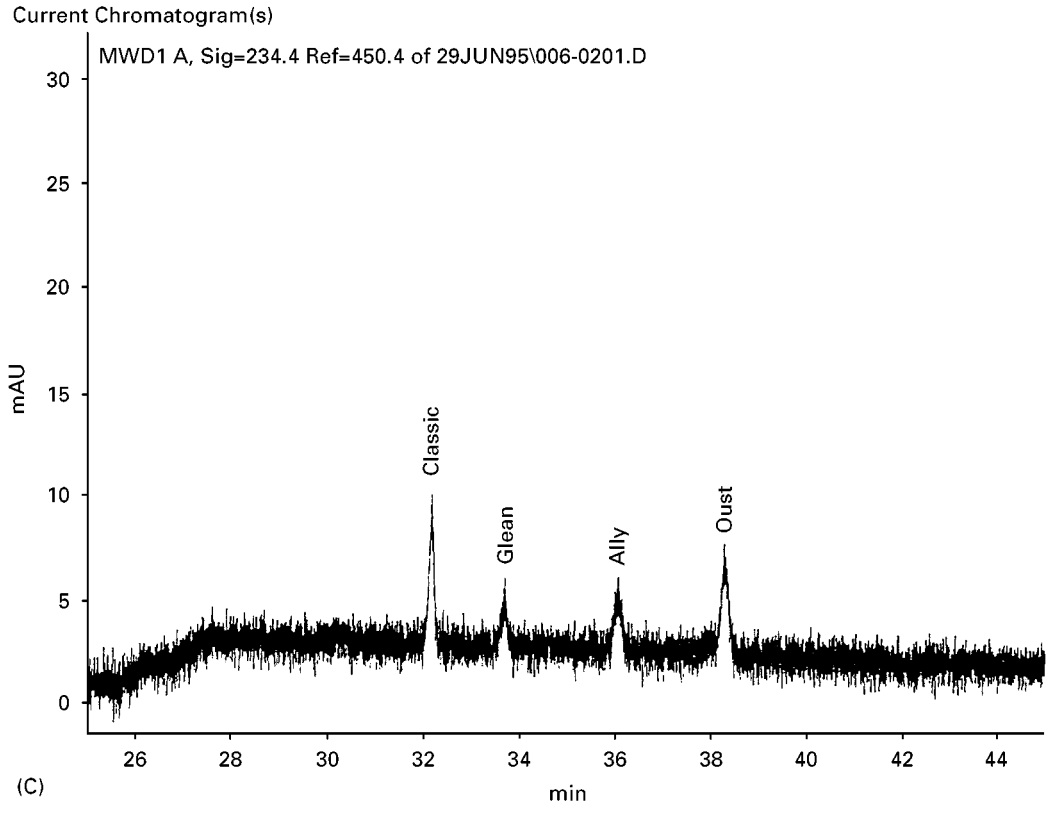

Figure 3 *Continued*

distance between the detector jet and bead. Standard calibration plots were compared for standards dissolved in acetone and into tomato and onion extracts. Slopes of the calibration curves were unchanged, suggesting an absence of matrix effects. Limits of detection for these organophosphorus insecticides ranged from 15 to 62 pg with methanol modifier.

The organophosphate pesticides chlorpyrifos, chlorpyrifos methyl, iodofenphos, leptophod, methidanthion, tetrachlorvinphos, phosmet, and famphur were separated using microbore packed column SFC with mass spectrometric detection. The separation was achieved with an amino column and 2% 2-propanol modified carbon dioxide. Baseline resolution was achieved in approximately 7 min except for chlorpyrifos and chlorpyrifos methyl. A high flow rate (HFR) interface between the SFC system and the mass spectrometer allowed for the use of packed columns. CI spectra were collected using ammonia or 2-propanol as the reagent gas. 94 pg on-column detection of chlorpyrifos in the selected ion monitoring mode yielded a S/N ratio of 21. An extract of cherries spiked with this mixture of insecticides was analysed utilizing this method.

Sulfur chemiluminescence detection with capillary SFC has been demonstrated for the analysis of malathion, carbophenothion, dioxathion, fenitrothion, and methyl and ethyl parathion. Restrictor tip positioning in relation to the chemiluminescence chamber was found to have a large effect on the quality of the chromatographic separation.

The capillary SFC was carried out on a 3.5 m × 100 μm DB-5 fused silica column with pure carbon dioxide mobile phase. The analysis of malathion from a commercial formulation was demonstrated. Detection limits were compound specific for malathion; 4.5 mg or 77 pg s^{-1} was reported while 39 ng (65 pg s^{-1}) was demonstrated for methyl parathion.

Phorate, Di-Syston, malathion, and ethion were investigated using capillary SFC and a microwave-induced plasma for detection. The 10 m × 50 μm, SB-cyanopropyl-50 capillary SFC column was used to introduce the pesticide samples into the plasma, using nitrous oxide as the mobile phase. Detection was conducted at the sulfur line. Baseline disturbances with both positive and negative slopes were observed at differing helium flow rates. An optimum helium flow rate for sulfur line monitoring was determined. With the use of nitrous oxide as a mobile phase, interference from CN-band emission was found with most of the lines investigated. Sensitivities were com-

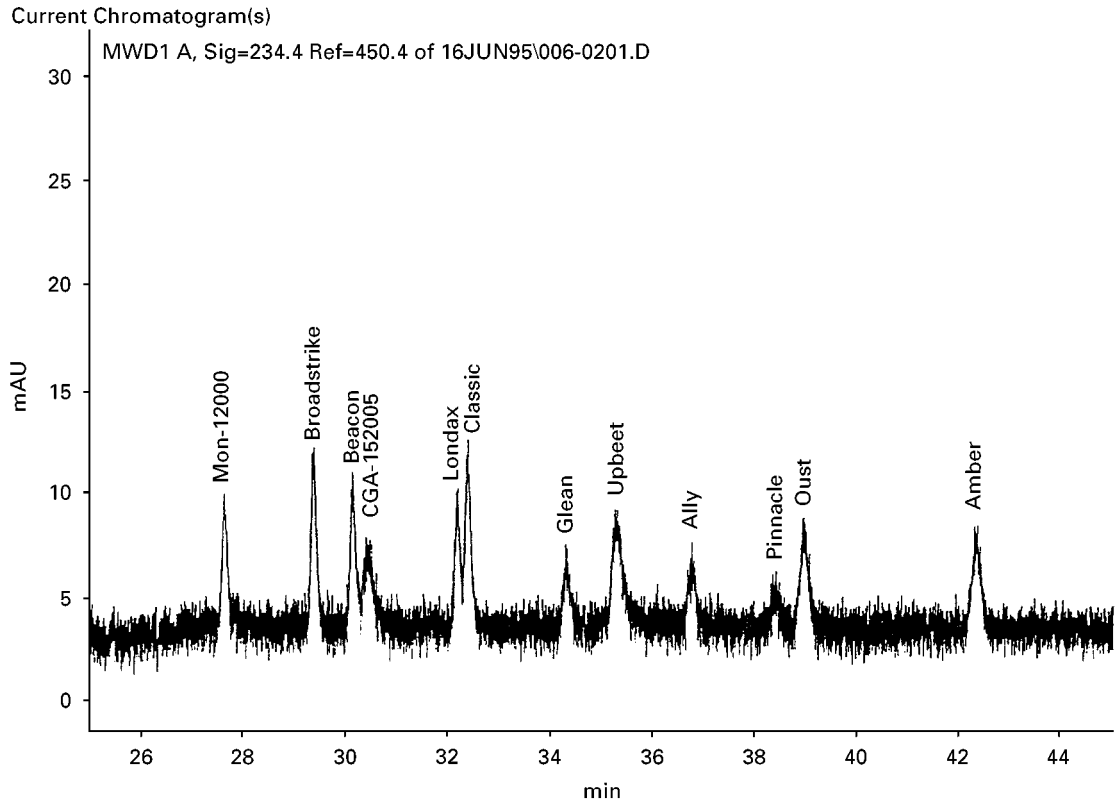

Figure 4 White Clay Creek water at 1.0 ppb.

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pound and mobile phase dependent. The sensitivity reported for sulfur with carbon dioxide as the mobile phase was 73 pg s^{-1}.

The separation of two organophosphorus insecticides (i.e. methidathion and chlorpyrifos) and a carbamate insecticide (i.e. carbofuran) was demonstrated by capillary SFC coupled with flame ionization and radio-frequency plasma detectors. When a FID was used, all three compounds were detected. When the RFP detector was used, either the chlorpyrifos or both the chlorpyrifos and the methidathion were detected, depending on whether the wavelength of chlorine emission or sulfur emission was being monitored. Short capillary columns, 2 to 3 m × 50 μm, and slow flow rates were used to prevent the introduction of too much mobile phase, and subsequent quenching of the plasma. The emission spectra of carbon dioxide and nitrous oxide did not show significant background or interference in the regions of interest for sulfur and chlorine.

Organochlorine Pesticides

A comparison between micro-column liquid chromatography and capillary SFC was conducted for the analysis of a series of organochlorine and other select pesticides from water samples. Small injection volumes in the SFC analysis limited the sensitivity, but the savings in analysis time was significant, 50 min compared to 10 h.

Acceptable recoveries of 2,4,5-T, 2,4-D, p,p′-DDD, methoxychlor, atrazine, dioctylphthalate, pyrene, pentachorophenol, cabazole, and hexachlorobenzene were demonstrated for the microbore LC isolation method using UV detection. The capillary SFC separation with a 10 m × 50 μm, SE-30 column and FID detection showed interfering peaks when SepPak cartridges were used. To eliminate this, water samples were preconcentrated through lyophilization. Both lake and river water samples were studied.

Detection limits of 0.64 pg were reported for Chlordane, a chlorinated hydrocarbon, Tri-allate, a thiocarbamate herbicide using capillary SFC and ECD detection. Arochlor 1254, and Arochlor 2565 were also separated and detected from the mixture. A 6 m × 50 μm capillary column and frit restrictors to provide back pressure to the system were used. Optimum make-up gas flow rate (10% methane/argon) was determined to be between 20 and 30 mL min^{-1}. Detector temperature affected the sensitivity in a compound-dependent manner. Separations of seven

thermally labile pesticides including metobromuron, fenitrothion, fenchlorphos, chlorbromuron, tetrachlorvinphos, tetradifon and diruon were accomplished in approximately 20 minutes.

Fourier transform mass spectrometry has been used for the detection of lindane, aldrin, DDE, dieldrin, DDD, DDT and methoxychlor in a mixture separated by SFC. Previously, this had been difficult due to the extreme difference in pressure requirements between the mass spectrometer and the supercritical fluid chromatograph. Relatively long capillary columns, 20 m × 100 μm, were used for this work. Typical chromatographic detection limits were in the low-nanogram range, although the sensitivity for aldrin and dieldrin was not as great due to the extent of fragmentation.

Carbofuran, α-BHC, γ-BHC, β-BHC, chlordane, and DDT were separated by capillary (6 m × 50 μm) SFC and detected with the use of a double focusing mass spectrometer. The interface developed was a direct heated probe. Spectra were collected in the negative-ion chemical ionization mode with methane as the reagent gas. The mass on column per compound was in the subnanogram range.

Chlordane was examined by capillary SFC and RFP detection using the emission wavelength for chlorine. The capillary SFC conditions are the same as those outlined in the organophosphorus section for methidathion and chlorpyrifos and a carbamate insecticide, carbofuran. The presence of DDT in milk was examined similarly. Using FID, milk extracts yield a complex chromatogram due to the triglycerides. Triglycerides are not detected by RPD, and a single peak for DDT is easily detected in milk. α- and β-BHC, chlordane, and methoxychlor were also separated and detected by RPD in the chlorine mode. Nitrous oxide was used as the mobile phase in this separation. Detection limits changed with the pressure of the capillary SFC system; higher detection limits were seen at higher pressures. Sulfur detection limits of 60 $pg\,s^{-1}$ were obtained at 100 atm, while this limit rose to 178 $pg\,s^{-1}$ at 400 atm.

Phthalimide Fungicides

Capillary SFC with electron-capture detection was unsuccessfully attempted for captafol and captan, although these compounds had been previously detected in SFC/FID experiments. This failure was attributed to the inability of these high melting compounds to be vaporized in the detector cavity of the ECD. The chromatographic mode is not the cause of this failed attempt as much as is the choice of detector.

Carbamoyloxime Insecticides

Aldicarb and methomyl, two carbamoyloxime insecticides, diflubenzuron, a benzoylurea and phenmedipham, a dicarbamate herbicide were separated on capillary columns (15 m × 50 μm ID) with a nitrogen-phosphorus detector. Similar separations of the four compounds were obtained when carbon dioxide and nitrous oxides were used as the mobile phase. Upon the addition of 1% THF as a modifier, changes in retention were observed, with the later eluting peaks, diflubenzuron and phenmedipham, showing larger changes in retention.

These same four pesticides were separated by capillary (10 m × 100 μm) SFC with FTIR (Fourier transform infrared) detection. The flow cell interface used was connected to the chromatographic column and to the restrictor by lengths of 100 μm fused silica capillary tubing, the same ID as the capillary separation column. 200 nL injection volumes of 5 $mg\,mL^{-1}$ of each component yielded spectral quality that was sufficient to provide structural information for these compounds. A 22 : 1 split ratio was utilized. 'Gram–Schmidt Plus' reconstruction techniques removed interference caused by changing density of the carbon dioxide with pressure programming. Spectral subtraction techniques were used to remove CO_2 features from the obtained IR spectra.

The use of dual-flame photometric detector with capillary SFC for the detection of the sulfur and phosphorus containing compounds: Oxamyl, parathion, chlorpyrifos, and Larvin. Modifications were made to the detector to make it compatible with supercritical mobile phases. The capillary SFC column was a 15 m × 75 μm i.d.. Detection limits were superior in the phosphorus mode, only slight baseline disturbance with pressure programming was noted over the range 50 to 200 atm (700 to 2900 psi). The detection limit of 0.5 ng (S/N = 2) was reported for parathion. In the sulfur mode more significant baseline disturbances were observed over the same pressure range. A baseline correction programme was used; even with this only a detection limit of 25 ng (S/N = 2) for benzo[b]thiophene was observed.

Pyrethrins

Six naturally occurring pyrethrin insecticides isolated from chrysanthemums were separated by capillary SFC. The pyrethrins were: cinerin I and II, jasmolin I and II, and pyrethrins I and II. Separation by a biphenyl column provided baseline resolution of the six compounds insecticides followed by on-line FTIR analysis. The separation of the six compounds by GC resulted in the degradation of pyrethrins I and II; therefore SFC/FTIR provided a way to analyse these

compounds without degradation. The SFC/FTIR spectra were able to distinguish between the structurally similar pyrethrins continuing to maintain the structural information provided by GC/IR analysis.

Future Developments

The future of SFC in pesticide and environmental analysis is not predictable. Currently more work is being reported in supercritical fluid extraction than in chromatography. However, research continues in a few locations with a few select individuals. The future of SFC seems to lie with chiral separations and pharmaceutical analysis and not with the environmentally oriented work presented in this review. This is not to suggest that the work presented here is without value. Advances into enhanced fluidity chromatography were most likely initiated from the work in SFC. As with other analytical techniques, success in one area leads to attempts in others.

Low waste generation in SFC is not enough to carry the technique into the arena where it can compete equally with LC and GC, the investment in training and equipment is too large a barrier to be overcome with what has fallen into the category of being a 'niche' technique. Liquid and gas chromatography are well cemented into environmental analysis. SFC of pesticides will continue to go beyond academic exercises to solve real problems, but most likely only when LC and GC do not yield a satisfactory result.

Further Reading

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Thin-Layer (Planar) Chromatography

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Introduction

Pesticides are a group of chemicals designed for killing weeds, pest control and plant growth regulation. They are poisons by design. Some of them also demonstrate carcinogenic potential and/or teratogenic activity. Pesticides (or products of their transformation in the environment) can penetrate soil, water, air and also food and fodder. As a result, pesticides are currently present in all parts of the environment. Many of them undergo degradation, but others are persistent and may accumulate in the food chain.

Development of residue analysis for pesticides is driven by toxicological purposes or by the need to identify residues. In the first case, the compounds are identified as being potentially hazardous to human health or to the environment. In the second case, the determination of residues is mainly aimed at inspecting and monitoring of food or environmental samples. Thin layer chromatography (TLC), including its modern developments (high-performance adsorbents, application of new, automatic techniques of spotting and development of chromatograms, spray-on technique of sample application), is still used for such analyses, especially in combination with selective biochemical detection methods or multidimensional methods although gas chromatography and high-performance liquid chromatography with selective detectors (ECD, NPD, AED or MS) are more important.

There is now a considerable literature describing pesticide analysis by TLC, with environmental and food monitoring, generally being the main aim in such